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Quantitative And Ultrastructural Studies Of Testicular Tissue Used In Cases Of Icsi (Intracytoplasmic Sperm Injection)

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Abstract

Background: Search for effective and acceptable samples in cases of intra- cytoplasmic sperm injection (ICSI) and for improvement of the % of success must be supported by examination of human testicular tissues which are used in this advanced technology.

Material and methods: In this study one hundred human testicular biopsies were obtained from Al- Hussein University Hospital, Department of Urology. Cases in this study were classified into the following groups:

1- Group I: (Control group) includes five cases of normal fertile persons. Patients consent was performed before sampling.

2- Group II: Includes 35 cases of infertile obstructive azoospermic patients. All of the rest of cases (60) were considered infertile non obstructive azoospermic cases and were included in both the third and the fourth groups.

3- Group III: Includes 20 cases of infertile non obstructive positive patients.

4- Group IV: Includes 40 cases of infertile non obstructive negative patients.

Cases in this group were either of **normal sized testis** or of **hypoplastic testis.**

The quantitative studies were done by using Image analyzing microscope and include the followings:

1- The thickness of the tubular basal lamina.

2- The thickness of spermatogenic cell layers.

3- The diameter of the seminiferous tubules

Results: In the **CONTROL GROUP** samples, it was noticed that testicular tissues were formed of two main components:

1- Tubular component, which is mainly formed of seminiferous tubules.

2- Interstitial compartment which is located between the seminiferous tubules.

Cases in **GROUP II (OBTRUCTIVE AZOOSPERMIA)** showed the following changes:1- No changes in the growth and development of the spermatogenic cells. 2- Changes in the vascular state of the tubule in the form of vascular dilatation and congested inter tubular capillaries 3- The number of the Sertoli cells was distinctly decreased 4-At the level of ultra structural examination, it was observed intra- cytoplasmic vacuolization in the Sertoli cells .

In **GROUP III (Non obstructive positive azoospermia)** showed the following changes: 1- The seminiferous tubules were more or less of normal structure and the number of the spermatogenic layers was normal and its lumen contained a considerable number of viable sperms. Other cases showed that the number of the spermatogenic cell layers was distinctly reduced 2- Ultra structural changes were noticed in the increased number of both myofibroblast and myoid cells in the basal lamina. 3- Leydig cells were decreased in number 4- In most of the cases the tubular lumen contains a considerable number of sperms.

Cases of **non obstructive negative azoospermia (GROUP IV A – Arrested spermatogenesis)** the testes were of normal size, The obtained data in this groups were: 1- The size of the testicular tubule was more or less within the normal but some of the tubules were of reduced sizes. 2- It was noticed that there was a distinct decrease in the spermatogenic cell layers. 3- No sperms were detected in the lumina of the tubules .4- The tubular basal lamina was thickened. 5- Leydig cells were few in number 6- The lumen of the tubules showed some spermiophage cells.

In Cases of GROUP IV- B (Normal sized testis SCOS) showed the following: 1-The testicular tubules were observed of moderate size. 2- No spermatogenic cells were identified and the tubules are only lined with Sertoli cells. No sperms were seen 3- The surrounding basal lamina was thickened and splitted to many layers.

GROUP IV – C (Normal sized testes with mixed atrophy) the findings were variable and showed the following: 1-Some tubules were of the arrested type of spermatogenic activity 2- In the same examined sections of the arrested spermatogenic activity suggesting the picture of **SCOS**.

Cases of this **GROUP IV - D** Hypoplastic testis were characterized by decreased volume of the testes with definite hypoplasia.

Quantitative changes, were in the form of variation in the thickness of the basal lamina and the mean thickness of the germinal epithelium as well as the luminal content of the sperms.

Conclusions: It could be concluded that structural changes in non obstructive azoospermia may explain failure rate in cases of ICSI, so we must find another way to select sperms used in this technique.

Review And Aim Of Work:

Infertility is an inability or reduced ability to induce a pregnancy within a reasonable period of trying, usually 12 months **(9)**. Infertility results from female disorders (anovulation, tubal obstruction, or other pathology) in about 30 %, a male disorder in 30 %, and disorders in both partners in 30 %. No abnormalities are found in about 10% **(21)**. Because male and female factors frequently coexist, both partners of the infertile couple are investigated and managed together.

Azoospermia occurs in between 5% and 20%, while in the general population, it is about 2% of infertile men. Azoospermia may be caused by obstruction of the extra testicular ductal system (obstructive azoospermia) or defects in spermatogenesis (non obstructive azoospermia) **(26)**.

The advancement in human in vitro fertilization **(IVF)** technology increases the options for treatment of infertile patients and may give the hope for some cases of azoospermic group of infertility.

Since the birth of Louise Brown in July 1978, in vitro fertilization **(IVF)** has proven to be an efficient treatment to alleviate female factor infertility, especially tubal infertility **(19)** and **(25)**. In subsequent years **(IVF)** was also successfully applied in couples with unexplained infertility, with male infertility as well as endometriosis **(19)**. At the end of the 1980s several procedures of assisted fertilization were developed and applied in couples where conventional IVF could not be used, including Intracytoplasmic sperm injection **(ICSI)**.

Research to develop an effective and being acceptable in cases of (ICSI) and for improvement of the % of success must be supported by examination of human testicular tissues which are used in this technology **(11)**. Also it is needed to understand the bases of testicular structure and evaluating its microscopic changes in different cases which are used in cases of (ICSI).

In this study it was planned to add a new parameter in evaluating and managing cases of infertility especially those of azoospermia. The microscopic examination of testicular tissue morphologically as well as quantitative evaluation of the obtained results could be of value in managing this group of patients. So, clinical and microscopic evaluations in this study could be of complementary importance and may increase the incidence of success.

Material And Methods:

In this study one hundred human testicular biopsies were obtained from Al-Hussein University Hospital, Department of Urology.

Evaluation of all Azoospermic cases in this study was performed through three main parameters:

1- Clinical examination of the cases for diagnosing the nature of each case, if it is of the obstructive or non obstructive type **(11)**.

2- Microscopic examination of the testicular biopsies for visualization of the sperms. Case was considered positive in the presence of the sperms and negative in its absence.

3- Microscopic examination of the prepared sections for detection of any structural changes in the testicular samples.

Cases in this study were classified into the following groups:

1- Group I: (Control group) Includes five cases of normal fertile persons The control samples were obtained from palliative orchidectomy(**11**) .Patients consent was performed before sampling

2- Group II: Includes 35 cases of infertile obstructive azoospermic patients.

All of the rest of cases (60) were considered infertile non obstructive azoospermic cases and were included in both the third and the fourth groups.

3- Group III: Includes 20 cases of infertile non obstructive positive patients.

4- Group IV: Includes 40 cases of infertile non obstructive negative patients. Cases in this group were either of normal sized testis or of hypoplastic testis and were subdivided into the following subgroups:

A - Cases with normal sized testes (30) infertile patients:

-Group IV a: Normal sized testis with arrested spermatogenesis.

-Group IV b: Normal sized testis with SCOS **(14)-(19).**

(Sertoli Cell Only Syndrome)

-Group IV c: Normal sized testis with mixed atrophy.

B - Cases with hypoplastic testes (10) infertile patients:

-Group IV d:This subgroup was of patients with hypoplastic testis

The testicular samples were prepared for histological, electron microscopic examination and finally for quantitative analysis.

A - Histological techniques: Testicular biopsies were prepared for examination of semi thin section and staining with toluidine blue.

B-Electron microscope study **(5)**

Specimens of testicular tissue, about 1mm, were fixed just after obtaining the biopsy. The first fixative used was glutraldehyde while the second fixative was 1% osmium tetroxide .Dehydration was done in ascending grades of ethyl alcohol , 50% , 70% , 90% and 100% .

The specimens were next impregnated in the (Epon) for forming capsules. Ultrathin section were cut by ultramicrotome and mounted on non coated grids, then stained with 2% uranyl acetate and lead citrate and examined by the electron microscope.

C- Quantitative studies

Using Image analyzing microscope different quantitative analysis was performed and includes the followings:

1- The thickness of the tubular basal lamina.

2- The thickness of spermatogenic cell layers.

3- The mean tubular diameter

The obtained results were tabulated and statistically analyzed using the studendt's T statistical test.

Results:

A- Structural changes (Semi thin, Plates 1 , 2) (Ultra structure, Plates 3, 4, 5).

It was noticed that sections of control human testicular biopsies were formed of two main components:

1-Tubular component, which is mainly formed of seminiferous tubules.

2-Interstitial compartment, which is located between the seminiferous

tubules. The results showed that the tubular compartment is formed

of the following cellular elements:

A- Spermatogenic cells:

B- Sertoli cells:

C- Peritubular cells:

The Primary spermatocyte was the largest of all the spermatogenic cells and by the electron microscopic examination it was characterized by its large vesicular nuclear appearance.

The spermatid was seen close to the tubular lumen and characterized by its spherical nucleus, by the electron microscope the acrosomal vesicle was observed especially in its late stage of development

Cases in group II (OBTRUCTIVE AZOSPERMIA) showed the following,

1- Morphologically no changes in the growth and development of the spermatogenic cells. Primary spermatocytes were noticed with its mitotic state in the prophase stage. The tubular lumen was full with many healthy sperms.

2- Changes in the vascular state of the tubule in the form of vascular diltation and congested intertubular capillaries

3- The number of the Sertoli cells was distinctly decreased.

4- At the level of ultra structural examination ,it was observed intra-cytoplasmic vacuolization was observed in the Sertoli cells mainly in the perinuclear area

Examination of sections in testicular tissues in group III (**Non obstructive positive azospermia)** showed the following microscopic data

1- The microscopic structure of the seminiferous tubules was variable in the different cases of this groups , Some cases the tubule was more or less of normal structure and the number of the spermatogenic layers was normal and its lumen contained considerable number of viable sperms.

Other cases showed that the number of the spermatogenic cell layers was distinctly reduced and the basal layers were separated from the basal lamina of the tubules. Degenerative vacuolization was noticed in the apical cell layers

2- The wall of the intertubular blood vessels was thickened and collagen deposition was increased in its wall, some of the vessels were occluded and its luminal epithelial cells were hypertrophied

3- Ultra structural changes were noticed in the increased number of both myofibroblast and myoid cells in the basal lamina.

4- Leydig cells were decreased in number at both levels of examination in were demonstrated semithin and ultrathin sections.

5-In most of the cases the tubular lumen contains a considerable number of sperms.

Cases of non obstructive negative azoospermia (Group IV A) were of normal size , all the obtained results in this group showed a case of arrested spermatogenesis. The obtained data in this group are:

1- The size of the testicular tubule was more or less within the normal level but some of the tubules were of reduced sizes.

2- It was noticed that there was a distinct decrease in the spermatogenic cell layers. Spermatogenic layers were arrested at the level of primary spermatocyte and no other layers were detected. No mitotic changes were observed.

The spermatogonia were seen with its both types resting on the basal lamina of the tubules.

3- No sperms were detected in the lumina of the tubules.

4- The tubular basal lamina was thickened by increased collagen bundles deposition.

5- The intertubular tissue showed affected blood vessels in the form of vascular occlusion and thickening of its wall. Some cases showed mild dilatation in the blood vessels. Leydig cells were few in number and showed mild degenerative changes.

6- The lumen of the tubules showed some spermiophage cells, such cell was irregular in shape and contains multiple nuclei .The cytoplasm showed faint acidophilic reaction.

Cases of group **IV B (Normal sized testis SCOS)** Showed the following findings:

1- The testicular tubules were observed of moderate size and surrounded with multilayered basal lamina.

2- No spermatogenic cells were identified and the tubules are only lined with Sertoli cells. Sertoli cells were observed to be tall with its intended basal nuclei. Cytoplasmic vacuolization was also observed.

3- No sperms were seen in the tubular lumens.

4-Intertubular tissue contains many blood vessels with characteristic thick wall and nearly occluded lumen. The endothelial lining was hypertrophied and seen in some vessel to be multiplied.

5- The surrounding basal lamina was thickened and splitted to many layers and in some tubules was forming knobs like structure invading the tubular cavity.

Cases of this group **(Normal sized testes with mixed atrophy)** were of variable findings because they include both results of arrested spermatogenesis and those of SCOS.

The results are the following:

1-Some tubules were of the arrested type of spermatogenic activity in the form of:

a- Reduced number of spermatogenic cell layers.

b- Absence of luminal sperms.

c- Reduced size of the tubules and irregularities in its border.

d- Increased thickness of the basal lamina.

e- Dilated vessels in the intertubular spaces and increased thickness of its lining epithelium.

f- At the level of both semithin sections and ultrastructural sections no cells were detected indicating spermatogenic activity.

h- Marked reduction in the number of leydig cells.

2- In the same examined sections of the arrested spermatogenic activity it was observed findings that suggesting the picture of SCOS represented in the following data:

a- The tubules were only lined by Sertoli cells with its intended nucleus.

b- No spermatogenic cells were observed as well as luminal sperms.

c- Vascular occlusions in the intertubular spaces were also observed.

d- Leydig cells were markedly decreased in number.

Cases of this group were characterized by decreased volume of the testes with definite hypoplasia. This finding was reflected on the microscopic characters of sections in this group are as follows:

1- The total size and volume of the tubular cross sections were decreased with very narrow central lumen, in some tubules it was nearly obliterated.

2- Marked reduction in the spermatogenic cell layers, and no sperms were detected in the tubular lumen.

3- Some tubules showed few Sertoli cells which were characterized by degenerative changes at the level of ultra structural examination.

4- The testicular tubules were surrounded by very thick basal lamina. Intertubular fibrosis was observed and increased intertubular spaces.

5- Reduced number of Leydig cells and in some specimens they were absent.

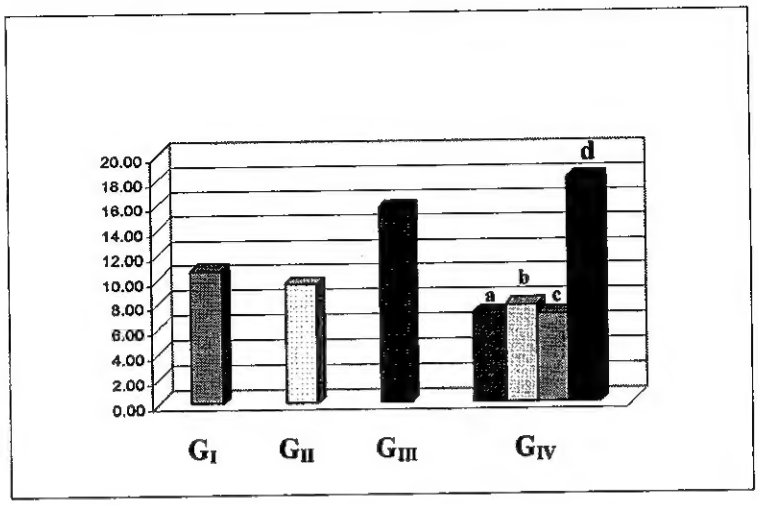
B- Quantitative changes

The main quantitative changes are illustrated in histographs (1-2-3) and can be summarized in the following items:

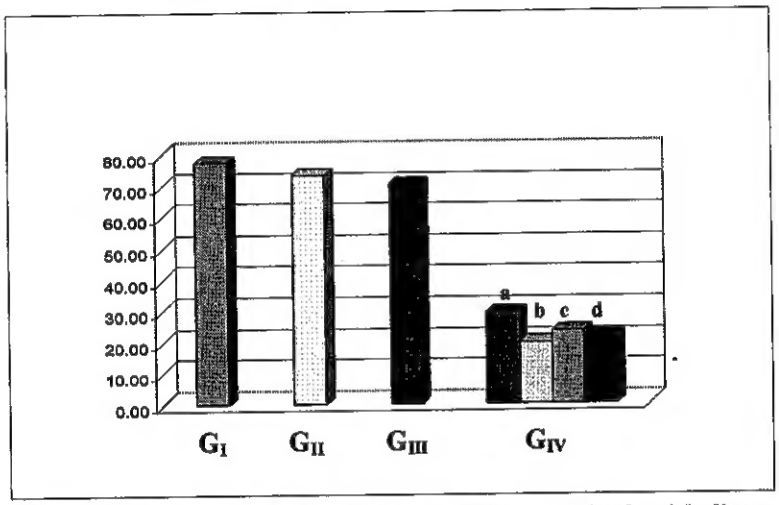
1- No changes in the **thickness of the basal lamina** in group II. The thickness showed marked increase in the group III while in the group IV showed variable changes.

2- No significant changes in the **thickness of the germinal cell layers** in both the second and the third groups. Significant decrease in that thickness in the fourth group and reach its maximum decrease in the subgroup(IV d).

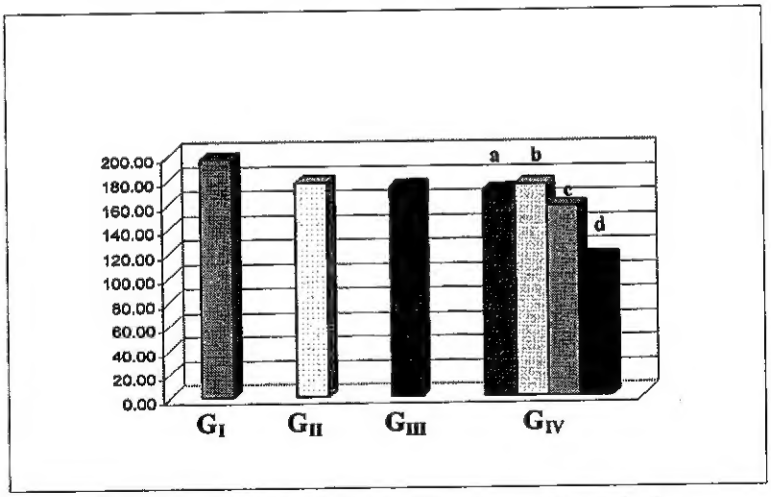
3- No significant changes in the **mean tubular diameter** in both groups II and III . No changes in both subgroups IV a and b but there was mild decrease in subgroup IV c and a marked decrease in subgroup IV d.



(Graph.1) Changes in the thickness of the basal lamina

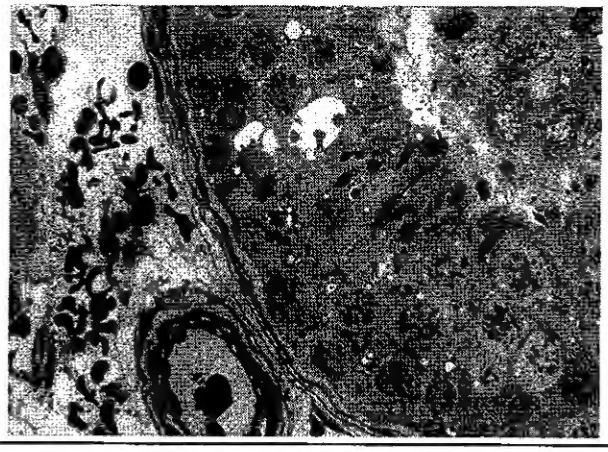


(Graph.2) Changes in the thickness of the germinal epithelium

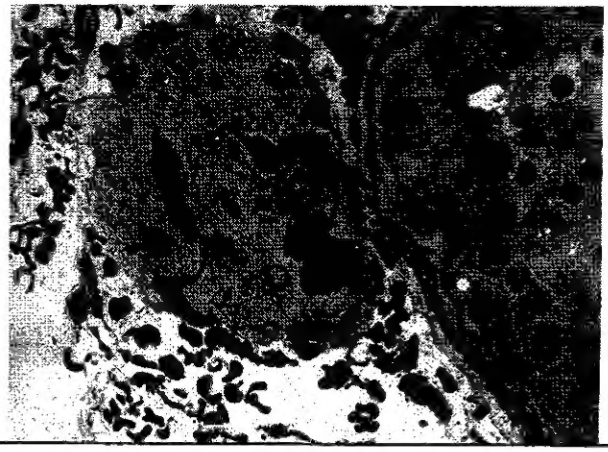


(Graph.3) Changes in the diameter of the tubules

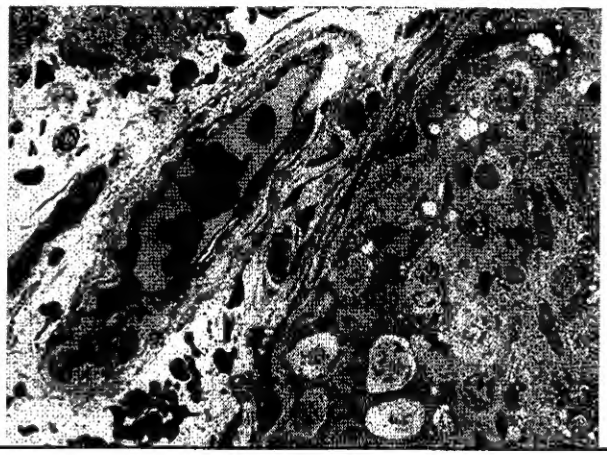
G = Group



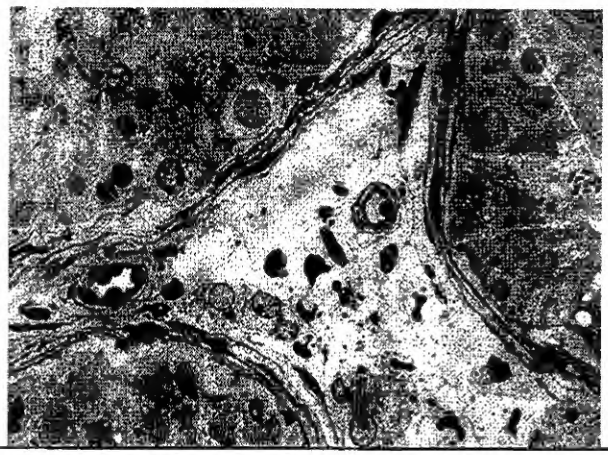
(Fig .1) Photomicrograph of semithin section in human control testicular tissue, **(Group I)** shows normal basal lamina with myoid cells , Sertoli cells. spermatogenic cells and sperms are seen . (Toludine blue, X1000)



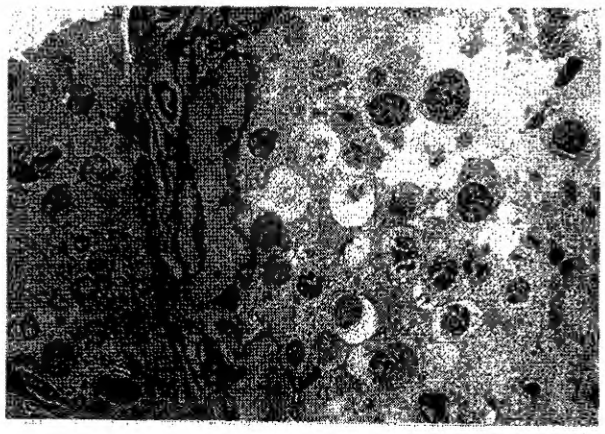
(Fig .2) Photomicrograph of semithin section in human control testicular tissue, **(Group I)** shows normal Leydig cells (Toludine blue, X1000)



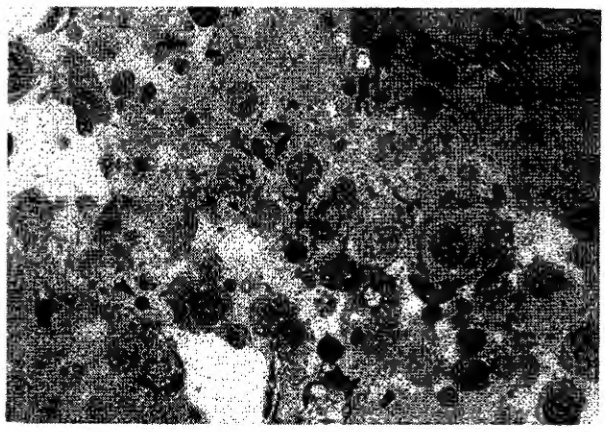
(Fig .3) Photomicrograph of a semithin section in human testicular tissue, **(Group II)** shows dilated and congested intertubular blood vessels (Toludine blue stain X 1000)



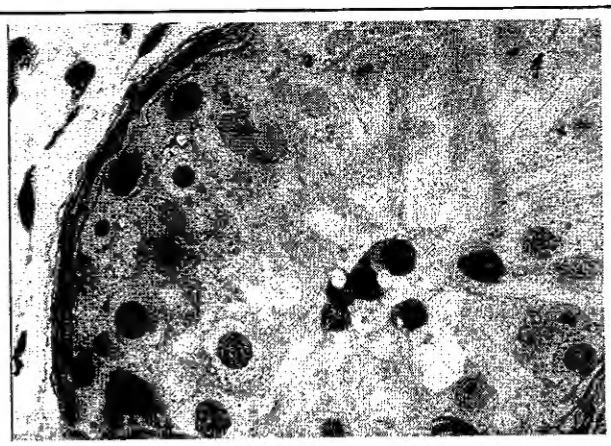
(Fig .4) Photomicrograph of a semithin section in human testicular tissue **(Group II)** shows interstitial tissue with little number of Leydig cells, myoid cells (Toludine blue stain X 1000)



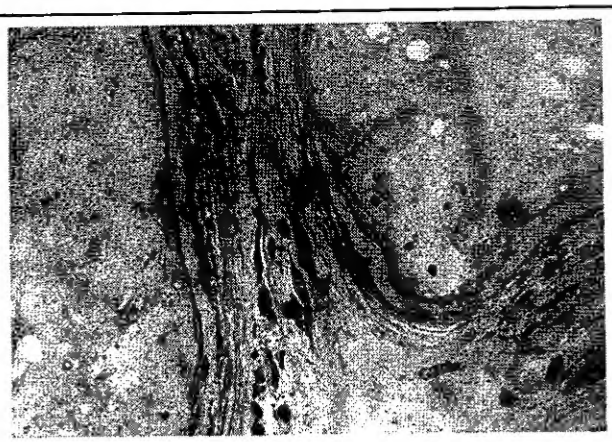
(Fig .5) Photomicrograph of semithin section in human testicular tissue, **(Group III)** shows marked thickened basal lamina, many primary spermatocyte in the prophase stage ,intertubular tissue contains leydig cells (Toludine blue stain X1000)



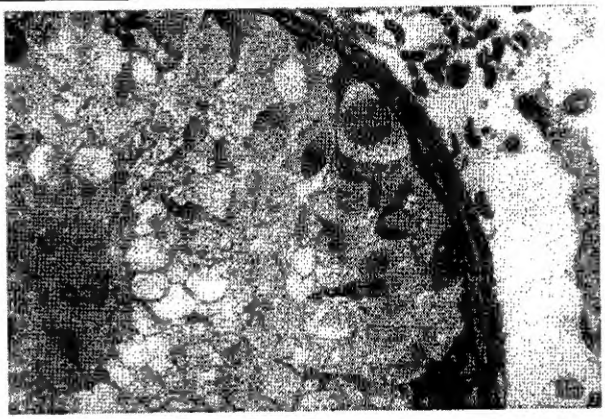
(Fig.6) Photomicrograph of semithin section in human testicular tissue **(Group III)** shows many sperm heads with mitotic figures in 1ry spermatocytes. (Toludine blue stain X1000)



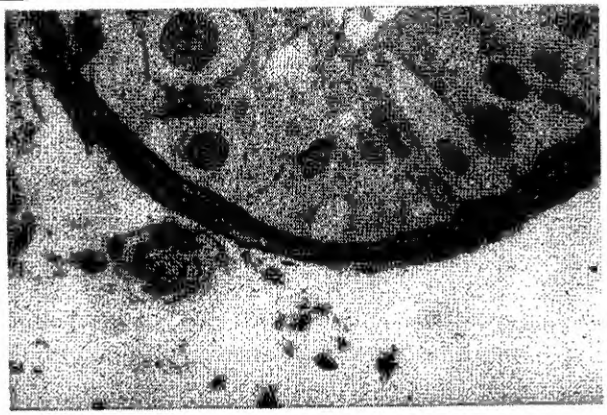
(Fig .7) Photomicrograph of semithin section in human testicular tissue, **(Group IVA)** shows arrested spermatogenic cells , lumen free of sperms. (Toludine blue, X1000)



(Fig .8) Photomicrograph of semithin section in human testicular tissue **(Group IVB)** shows two seminiferous tubules with marked thickness of the tubular wall. Only spindle shaped fibroblasts in between (Toludine blue stain X 1000)



(Fig .9) Photomicrograph of semithin section in human testicular tissue, **(Group IVC)** shows thickened basal lamina . (Toludine blue stain X 1000)



(Fig .10) Photomicrograph of semithin section in human testicular tissue, **(Group IVD)** shows thick basal lamina and Sertoli cells with its indented nucleus . (Toludine blue stain X1000)

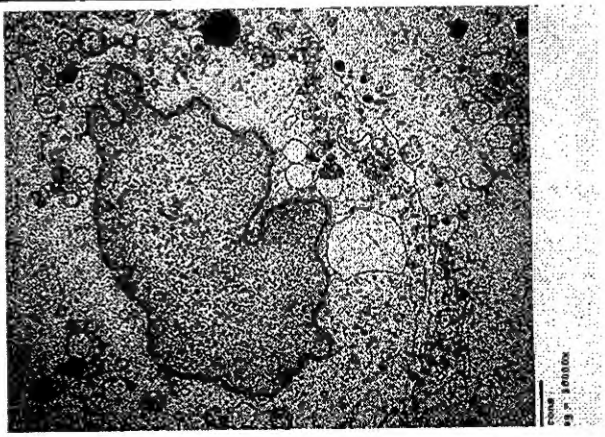
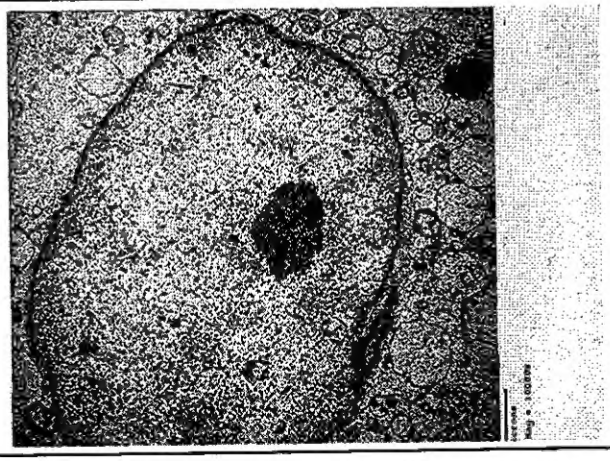
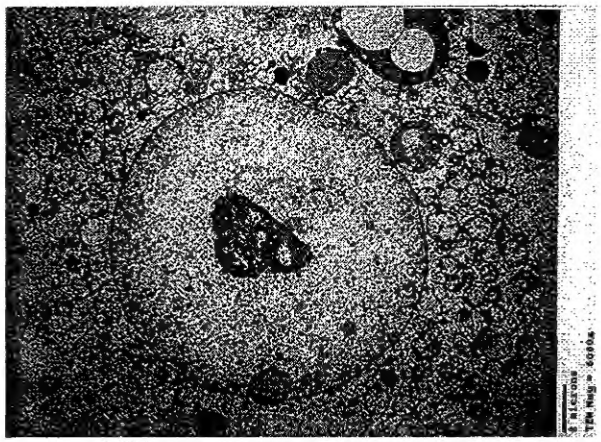


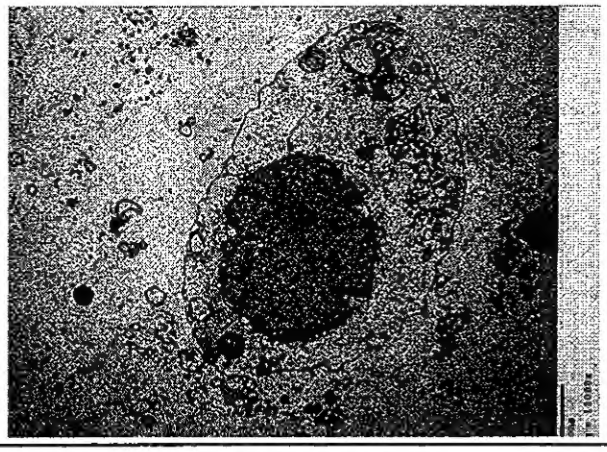
Fig .11) Photomicrograph of TEM in human control testicular tissue **(Group I)** shows normal intended nucleus of Sertoli cell. (X 10000)



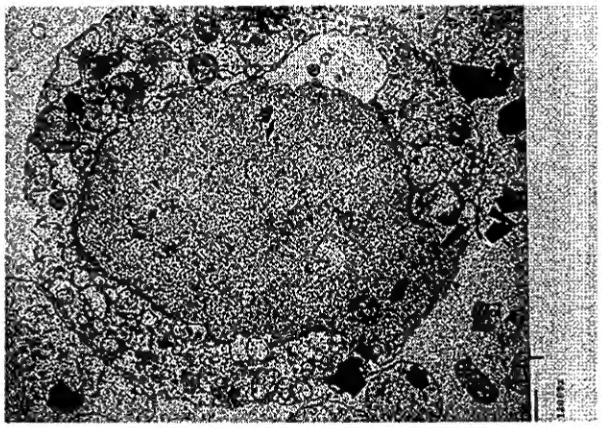
(Fig .12) Photomicrograph of TEM in human control testicular tissue **(Group I)** shows spermatogonia type A. (X 10000)



(Fig .13) Photomicrograph of TEM in human control testicular tissue **(Group I)** shows spermatogonia type B. (X 6000)

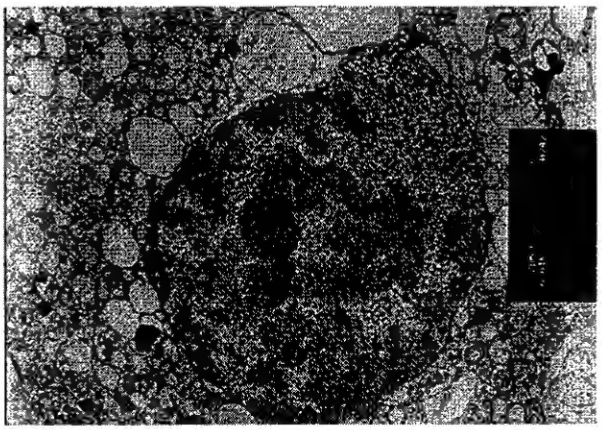


(Fig .14) Photomicrograph of TEM in human control testicular tissue **(Group I)** shows round early spermatid cell. (X 12000)

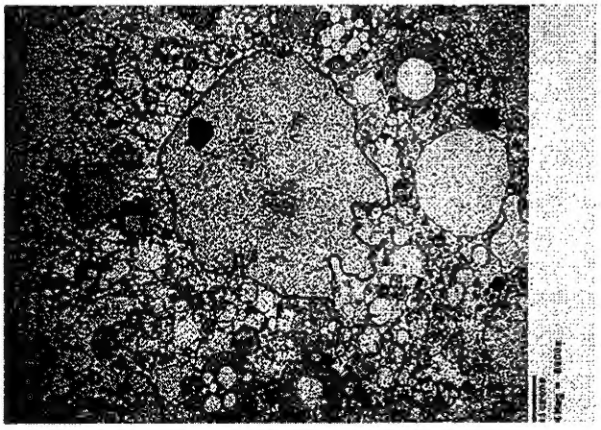


(Fig .15) Photomicrograph of TEM in human control testicular tissue **(Group I)** shows late spermatid (X 12000)

(Fig .16) Photomicrograph of TEM in human control testicular tissue **(Group I)** shows head of sperm (X 8000)

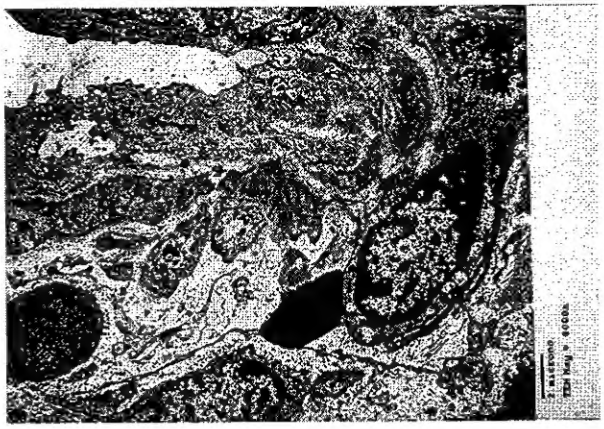


(Fig .17)TEM Photomicrograph of human testicular tissue **(Group II)** shows active 1ry spermatocyte. (X 10000)

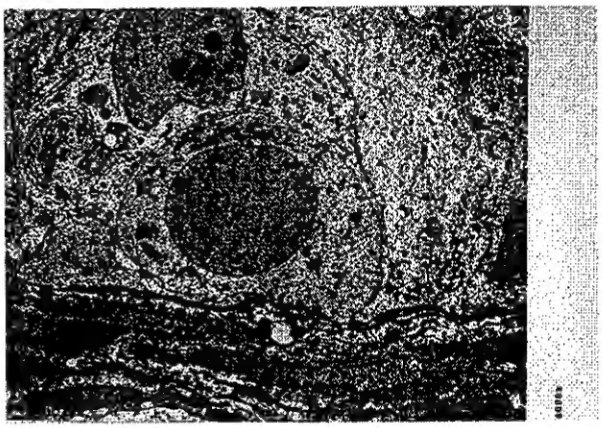


(Fig .18)TEM Photomicrograph of human testicular tissue **(Group II)** shows normal intended nucleus of Sertoli cells with many intracytoplasmic vacuoles (X 8000)

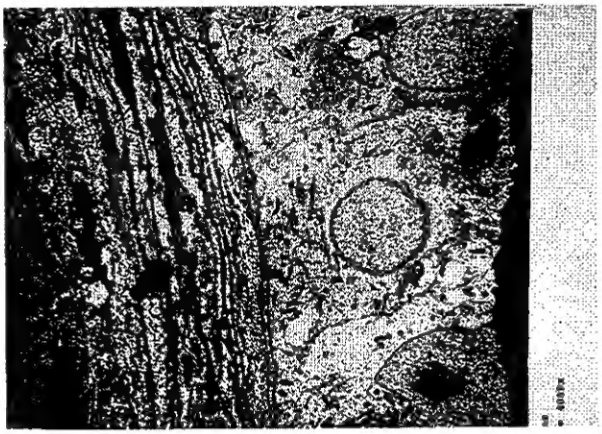
(Fig .19)TEM Photomicrograph of human testicular tissue **(Group III)** shows multiple layers of fibroblast cells with spindle shaped nucleus and myoid cell (X 10000)



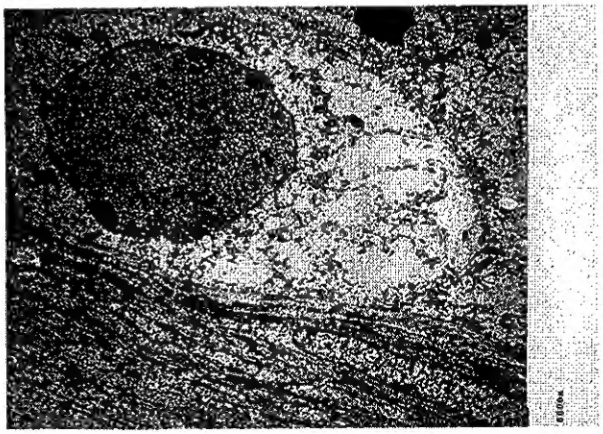
(Fig .20)TEM Photomicrograph of human testicular tissue **(Group III)** shows head of a sperm. (X 6000)



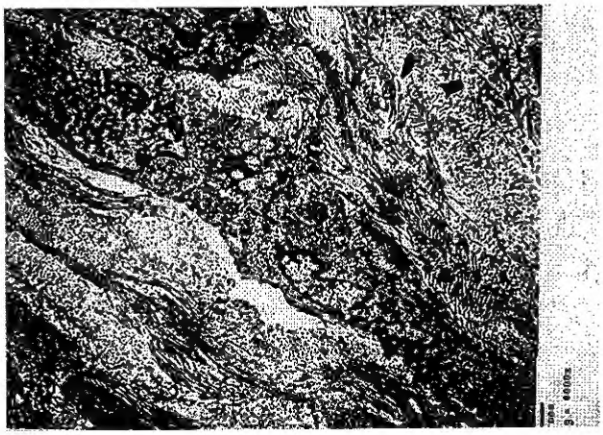
(Fig .21)TEM Photomicrograph of human testicular tissue **(Group IVA)** shows thick basal lamina, spindle shaped myoid cells and myofibroblast, part of Sertoli cell with indented nucleus . (X 4000)



(Fig .22)TEM Photomicrograph of human testicular tissue **(Group IVB)** shows thick basal lamina. Sertoli cells appear vacuolated and loss of characteristic nuclear indentation. (X4000)



(Fig .23)TEM Photomicrograph of testicular tissue **(Group IVC)** shows multiple layers of basal lamina, spindle shaped myoid cells . one of spermatogonia type A with its peripheral nucleolus and marked degeneration and vacuolation (X 6000)



(Fig .24) TEM Photomicrograph of human testicular tissue **(Group IVD)** shows thickened basal lamina formed of many layers with fibroblast (X 8000)

Discussion

Intracytoplasmic sperm injection (ICSI) was developed in the early 1990s. In ICSI, a single spermatozoon is microinjected into the oocyte after passage through the zona pellucida and oocyte membrane.

The outcome of ICSI using non – ejaculated sperm may be influenced by various factors, including the etiology of azoospermia and the surgically retrieved sperm source, sperm status being fresh of after crypreservaton (3).

This study aiming to evaluate the morphological and ultra structural changes of the testicular tissue in azoospermic patients with primary infertility that is prepared for ICSI. The human testicular samples were obtained from Islamic Center for Reproduction and Urology Department (Al-Hussein Hospital). Patients consent was applied before taking the biopsy. The sample was obtained by open biopsy **(6)**. This method is important to support our histological testicular findings.

It must be noticed that the strongest indicator for finding sperm for ICSI is testicular histological study.

Our results showed that the seminiferous tubules in the control group were rounded with lumen full with sperms. **(15)**. The ultra structural examination showed that the tunica propria was formed of an acellular zone of electron dense basal lamina while there is a cellular zone formed of both myoid cells and fibroblasts.
(10) and **(24)** stated the normal Sertoli cells are essential to participate in the formation of normal peri tubular tissue.

The peri tubular wall of obstructive azoospermic cases showed mild thickening and tubular enfolding, the same findings were obtained by **(4)** and **(18)**.

Sertoli cells in this study showed cytoplasmic numerous lipid droplets, phagolysosome and mild vacuoles, similar changes were reported by **(5)** and **(24)**.
The increased lipid droplets and Sertoli vocalization may be due to increase rate of phagocytosis of the aging sperms as a results of obstruction.

In cases of Sertoli only syndrome (SCOS) it was noticed that peri tubular wall was thick with increased amount of collagen fibrils.

The study showed premature sloughing of germ cells and this may be related to disturbed Sertoli-germ cell junction complexes, the same findings were reported by **(9)** and **(18)**.

The ultra structural changes in the SCOS showed different pathological changes where there was different forms of Sertoli cells, mature , immature and dysgenetic as well as involuting.**(2)** and **(20)**.

Cases of mixed atrophy showed thickening in the peri tubular wall, some tubules showed changes similar to SCOS while the others showed spermatogenic arrest **(13)**.

It was observed the presence of enlarged multinucleated giant cells in the spermatogenic region of the tubules in most cases. Multinucleated cells were observed in persons treated with testosterone **(1)** and normally seen in rabbits **(17)** mice and dogs **(2)**.

The quantitative changes in the thickness of the basal lamina may support that the disturbance in the membrane integrity may be the cause of degenerative changes in the tubule contents, the same finding was observed by **(22)** and **(8)**.

The changes in the mean thickness of spermatogenic cell layers in this study were observed by **(16)** and **(12)**.

The changes in the mean diameter of the tubules were in accordance with **(8)** , **(16)** and **(12)**.

Finally it could be concluded that structural changes in non obstructive azoospermia may explain failure rate in cases of ICSI, so we should find another way to select sperms used in this technique. Also the abuse of hormonal treatment may cause more harm than being of good effect so must applied only in needed cases.

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دراسة كمية ودقيقة لأنسجة الخصية المستخدمة في حالات الإخصاب المجهري

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تهدف هذه الدراسة إلى تقييم التركيب الميكروسكوبى لأنسجة الخصية للحالات المستخدمة فى الأخصاب المجهرى. وقد تم إستخدام مائة حالة من عينات الخصية البشرية من المركز الأسلامى للتكاثر وقسم المسالك البولية بمستشفى الحسين الجامعى وتم أخذ موافقة المرضى قبل أخذ العينات وقد تم تقسيم الحالات على النحو التالى: 1– المجموعة الأولى الضابطة حيث اشتملت على خمس حالات لأشخاص طبيعين قادرون على الأنجاب طبقا لتحليل السائل المنوى. وبالنسبه لباقى الحالات من الرجال الذين يعانون من عقم أولى ناتج من عدم وجود حيوانات منوية فى السائل المنوى وقد تم تقسيمهم إلى المجموعات التاليه:

2– المجموعة الثانيةوشملت حالات الأنسداد بالأنبوب المنوى وشملت خمس وثلاثون حالة.

3–المجموعة الثالثة وشملت حالات ليس بها إنسداد بالأنبوب المنوى وموجبة للحيوانات المنويةواحتوت على عشرون حالة.

4–المجموعة الرابعة وشملت حالات ليس بها إنسداد للأنبوبه المنويه ولكنها سالبة للحيوانات المنويه واحتوت على أربعون حالة تمت تقسيمها إلى:

ا– حالات تميزت بحجم طبيعى للخصية بعدد ثلاثون حالة تم تقسيمها إلى أربعة مجموعات فرعية.

ب– حالات بها ضمور فى حجم الخصية وشملت عشر حالات

وتم تحضير العينات تمهيدا لفحصها بكل من الميكروسكوب الضوئى والألكترونى وكذلك عمل التحليل الكمى والذى ركز على قياس سمك كل من الصفيحة القاعدية وتقدير سمك طبقة الخلايا الجرثومية وكذلك تقدير قطر الأنبوب المنوى. وقد دلت النتائج على حدوث تغييرات تركيبيه فى المجموعات المختلفة لهذه الدراسه.

ففى المجموعة الثانية لم يوجد إختلافات جوهريه إذا ما قورنت بالمجموعة الأولى بجانب إحتقان واضح فى الأوعية الدموية. وقد لوحظ ازدياد واضح فى سمك الصفيحة القاعدية. وعلى مستوى الفحص بالميكروسكوب الألكترونى لوحظ بعض التجاويف فى السائل الهلامى لخلايا سرتولى.

ولوحظ فى المجموعة الثانية إزدياد فى سمك الصفيحة القاعدية وازدياد واضح فى سمك جدر الأوعية الدموية كما لوحظ وجود أعداد مناسبة من ا لحيوانات المنوية.

فى المجموعة الثالثة وجد أن حجم الأنابيب المنوية قد يكون قريب من الطبيعى ولكن يوجد ضمور فى بعضها وكذلك كان هناك نقص واضح فى عدد الخلايا الجرثوميه ولم يلاحظ وجود للخلايا أرومة النطفة. وقد لوحظ أيضا وجود خلايا متعددة الأنوية وفى المجموعة الرابعة والأخيرة لوحظ أن حجم الأنابيب متوسط أو صغير وكذلك عدم وجود طبقات الخلايا الجرثومية حيث اشتملت الأنابيب على خلايا سرتولى فقط.

وقد دلت الدراسة الكميه على ازدياد سمك الصفيحة القاعدية فى كل من المجموعتين الثالثة والرابعة بجانب نقص واضح فى سمك طبقة الخلايا الجرثومية بالذات فى مجموعات المجموعة الرابعة. كما لوحظ تغيير واضح فى أقطار الأنابيب المنوية فى عينات المجموعة الرابعة.